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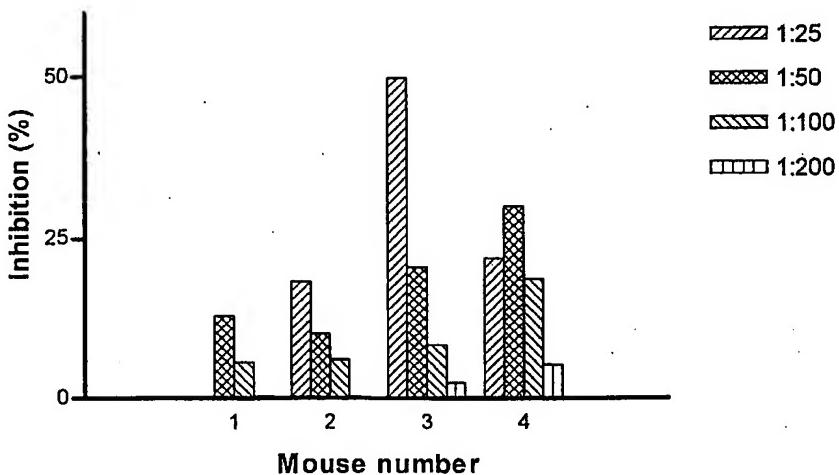
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(54) Title: PEPTIDE-BASED CYTOKINE/CHEMOKINE VACCINES AGAINST ALLERGY

IL-4 inhibition tests



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(57) Abstract: This invention relates to the methods and materials involved in the prevention and treatment of allergy. The inventors have identified a number of key peptides derived from the receptor binding sites of Th2 cytokines/chemokines such as interleukin (IL)-4, -5, -9, -13, -25, catoxin and TARC and from the cytokine/chemokine binding sites of their receptors such as IL-4R $\alpha$ . These peptides are made immunogenic by linkage to a carrier protein such as the Hepatitis B surface antigen (HBsAg) or the Hepatitis core antigen (HBcAg) via construction of a fusion protein or chemical methods to form a vaccine compound. The vaccine which consists of the vaccine compound and a human adjuvant can be used to down regulate allergic responses induced by over-expressed Th2 cytokines and chemokines. The vaccine itself or in combination with its DNA form induces autoantibodies which bind to cytokines/chemokines, thus reversing and preventing allergic responses for a long term.

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## Peptide-based cytokine/chemokine vaccines against allergy

### PRIOR APPLICATION INFORMATION

The present application claims priority on USSN 60/465,276, filed April 25,  
5 2003.

### FIELD OF THE INVENTION

The present invention relates generally to the field of immunization and vaccination.

Abbreviations used:

IL:	Interleukin(s)	HbcAg:	Hepatitis B virus core antigen
ILR:	Interleukin(s) receptor	HBsAg:	Hepatitis B virus surface antigen
mAb(s):	Monoclonal antibody(s)	CFA:	Complete Freund's adjuvant
ELISA:	Enzyme-linked immunosorbent assay	Fc $\epsilon$ RI:	High affinity receptor for IgE

10

### BACKGROUND OF THE INVENTION

Allergic or atopic diseases affect up to 42% of the population, especially children.<sup>1</sup> The "International Study of Asthma and Allergies in Childhood" has shown that Canada and other North American countries have a very high prevalence for asthma symptoms (19%), wheezing (31%), allergic rhinoconjunctivitis (46%) and atopic eczema (22%).<sup>2</sup>

Of these allergic disorders, asthma is most important. The incidence and prevalence rates of asthma and the rate of hospitalizations for asthma exacerbations are increasing.<sup>3-5</sup> Asthma is a chronic lung disease caused by inflammation of the lower airways that is characterized by recurring breathing problems. Inflammation results in narrowing of the airways and reduces airflow in and out of the lungs, making breathing difficult and leading to wheezing and coughing. Although asthma is multifactorial in origin, the inflammation process is believed to be a result of inappropriate immune responses to common aeroallergens in genetically susceptible individuals.

Over two decades ago, two T helper cell subsets, Th1 and Th2, were distinguished on the basis of their cytokine production patterns.<sup>6</sup> In mice, Th1 cells secrete IL-2, IFN- $\gamma$ , IFN- $\alpha$ , IFN- $\beta$ , and IL-12 (a cytokine made by antigen-presenting cells), whereas Th2 cells secrete cytokines such as IL-4, IL-5, IL-9, and

IL-13 (an honorary Th2 cytokine). Th1-type cytokines mediate delayed type hypersensitivity responses against intracellular pathogens and inhibit the Th2 responses. Th2 cytokines are relevant in humoral immune responses and all Th2 cytokines especially IL-4, IL-5, IL-9, and IL-13 have effects that are associated with atopy and asthma.<sup>7-10</sup> In addition, recently reported IL-25 may be an important mediator of allergy via up-regulation of IL-4, IL-5, and IL13<sup>11</sup>.

Below is the list of the Involvement of Th2 cytokines in allergy and asthma:

	Stimulation of IgE production	IL-4, IL-9, IL-13*
	Eosinophil, neutrophil, and mast cell infiltrates	IL-4, IL-5, IL-9, IL-13
10	Mastocytosis	IL-3**, IL-4, IL-9
	Goblet cell hyperplasia	IL-4, IL-5, IL-9, IL-13
	Th2 cell differentiation	IL-4
	Increased adhesion molecule expression	IL-4, IL-13
	Increased vascular permeability	IL-4, IL-5, IL-13
15	Airway hyperresponsiveness	IL-4? IL-5, IL-9, IL-13
	Smooth muscle hyperplasia	IL-4, IL-9, IL-13
	Subepithelial fibrosis	IL-4, IL-5, IL-9, IL-13

20 Chemokines – in particular, the eotaxin subfamily (e.g. eotaxin TARC and MDC) and their receptor CC chemokine receptor 3 – have also emerged as cytokines likely to be important in the regulation of allergic inflammation. Chemokines are potential leukocyte chemoattractants, cellular activating factors, 25 and histamine releasing factors. Extensive studies have demonstrated a central role for chemokines in the pathogenesis of allergic inflammation.<sup>10, 12</sup>

Because Th2 cytokines and chemokines play a central role in the development of allergic and airway inflammatory responses, limiting the production of these cytokines/chemokines or their accessibility to their respective receptors may control the pathogenic process. To date, many new therapeutic approaches have been used, but the inhibition of IgE and Th2 cytokines offers an especially useful approach in suppressing inflammation and preventing airway hyperresponsiveness in a broader, antigen-nonspecific fashion. These strategies

include administration of humanized monoclonal antibodies (mAbs) against these molecules or their receptors, soluble receptors, or mutated Th2 cytokines.<sup>9, 13, 14</sup>

Humanized mAbs against IgE, up-regulated by Th2 cytokines, have been successfully used in the treatment of allergy and asthma in many clinical trials.<sup>15, 16</sup>

- 5 However, most humanized or human mAbs against Th2 cytokines or chemokines such as eotaxin<sup>17</sup> and their receptors are still currently under development. Different approaches have been tested to block IL-4 functions resulting clinical improvements,<sup>18</sup> for example, using mAb to IL-4,<sup>19, 20</sup> mAb to its receptors, soluble receptors<sup>21, 22</sup> or IL-4 mutein. Similar approaches have also been investigated to  
10 block the pathogenic process induced by IL-13,<sup>23</sup> IL-9,<sup>24</sup> IL-5,<sup>25</sup> and eotaxin.<sup>17</sup> Although humanized or human mAbs against IL-13 or IL-9, and soluble human IL-13 receptors are currently under development, the effect of administration of a polyclonal anti-IL-13<sup>26</sup> or anti-IL-9<sup>27, 28</sup> has shown to attenuate airway hyperresponsiveness in mice, indicating the therapeutic effect of these mAbs.
- 15 Because the above reagents acting as passive blockers have significant disadvantages in the treatment of allergic diseases, active vaccines against over-expressed self-molecules have been reported to down regulate over-expressed self-molecules. Vaccination of ovalbumin-sensitized rats with constant domains two and three of rat IgE, coupled to a heterologous carrier protein, resulted in a  
20 profound decrease (90%) in serum IgE.<sup>29, 30</sup> Anti-IL-9 antibodies were persistently produced in mice vaccinated with mouse IL-9 coupled to ovalbumin.<sup>31</sup> The antibodies induced by coupling a self-molecule (whole or a significant portion) to a carrier protein may cross-react with other molecules with homogenous components and may bind to cell-bound IgE causing histamine release. IgE  
25 epitope-based vaccines have been recently reported in animal studies.<sup>32, 33</sup> These studies support the concept that active vaccination against over-expressed self-Th2 cytokines/chemokines is feasible, especially using peptide-based vaccines.  
30 The only patent found to be related to this invention (WO 02/070711, 2002)<sup>34</sup> teaches inducing mutations within a cytokine which correspond to sequence heterogeneities between species, thereby forming a chimeric protein which contains cytokine sequence regions from a plurality of species but should still fold properly. However, the antigenicity of the vaccine is weak. In animal studies, neutralizing antibodies could not be induced when their vaccine was mixed with

complete Freund's adjuvant (CFA), and were induced only when it was mixed with CpG oligodeoxynucleotides.

#### SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a reagent  
5 comprising:

- a cytokine or chemokine-derived peptide or a cytokine- or chemokine-receptor derived-peptide;
- a carrier protein; and
- an adjuvant.

10 According to a second aspect of the invention, there is provided a method of inducing an immune response in an individual, comprising:  
administering to an individual in need of such a treatment, an effective amount of a composition comprising:

- a cytokine or chemokine-derived peptide or a cytokine- or chemokine-receptor derived-peptide;
- a carrier protein; and
- an adjuvant.

15 According to a third aspect of the invention, there is provided a method of treating, ameliorating or preventing asthma comprising:  
20 administering to an individual in need of such a treatment, an effective amount of a composition comprising:

- a cytokine or chemokine-derived peptide or a cytokine- or chemokine-receptor derived-peptide;
- a carrier protein; and
- an adjuvant.

25 According to a fourth aspect of the invention, there is provided an expression system comprising:

- a nucleic acid molecule deduced from a peptide selected from the group consisting of: 6 or more consecutive residues of DITLQEIIKTLNSLT (SEQ ID No. 1, amino acids 4-18 of IL-4); 6 or more consecutive residues of EKETFCRAATVLRQFYSHH (SEQ ID No. 2, amino acids 41-59 of IL-4); 6 or more consecutive residues of QQFHRHKQLIRFLKRLDRNLWGLA (SEQ ID No. 3, amino acids 71-94); 6 or more consecutive residues of TLENFLERLKTIMREKYS

(SEQ ID No. 4, amino acids 108-125); 6 or more consecutive residues of EQKTLCTELTVTDIFA (SEQ ID No. 5, amino acids 19-34); 6 or more consecutive residues of AGLNSCPVKE (SEQ ID No. 6, amino acids 94-103 of IL-4); 6 or more consecutive residues of VPPSTALRELIEELVNITQ (SEQ ID No. 7, amino acids 4-5 of IL-13); 6 or more consecutive residues of MYCAALESLI (SEQ ID No. 8, amino acids 43-52 of IL-13); 6 or more consecutive residues of VAQFVKDLLLHLKK (SEQ ID No. 9, amino acids 92-105 of IL-13); 6 or more consecutive residues of KDLLLHLKKLFREGRFN (SEQ ID No. 10, amino acids 97-113 of IL-13); 6 or more consecutive residues of KIEVAQFVKDLLLHLKKLFREGRFN (SEQ ID No. 11, amino acids 89-113 of IL-13); 6 or more consecutive residues of SAIKEKTQRMLSGFC (SEQ ID No. 12, amino acids 58-71 of IL-13); 6 or more consecutive residues of NVSGCSAIEKTQRMLSGFC (SEQ ID No. 13, amino acids 53-71 of IL-13); 6 or more consecutive residues of PTLAGILDINF (SEQ ID No. 14, amino acids 4-11 of IL-9); 6 or more consecutive residues of TRYPLIFSRVKKSV (SEQ ID No. 15, amino acids 65-79 of IL-9); 6 or more consecutive residues of NALTFLKSLLEI (SEQ ID No. 16, amino acids 102-113 of IL-9); 6 or more consecutive residues of PASKCHCSANVTSCCLCG (SEQ ID No. 17, amino acids 23-40 of IL-9); 6 or more consecutive residues of CTRPCFSE (SEQ ID No. 18, amino acids 46-53 of IL-9); 6 or more consecutive residues of KNNKCPYFSCEQPCN (SEQ ID No. 19, amino acids 82-96 of IL-9); 6 or more consecutive residues of PTSALVKETLALLSTHRTLLIA (SEQ ID No. 20, amino acids 6-27 of IL-5); 6 or more consecutive residues of PTSALVKETLALLST (SEQ ID No. 21, amino acids 6-20 of IL-5); 6 or more consecutive residues of HRTLLIA (SEQ ID No. 22, amino acids 21-27 of IL-5); 6 or more consecutive residues of EERRRVNQFLD (SEQ ID No. 23, amino acids 88-98 of IL-5); 6 or more consecutive residues of TVERLFKNLSLIK (SEQ ID No. 24, amino acids 64-77 of IL-5); PVHKNH (SEQ ID No. 25, amino acids 36-41 of IL-5); 6 or more consecutive residues of LYQLVFLLSEAH (SEQ ID No. 26, amino acids 36-47 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of LLMDDVVSAD (SEQ ID No. 27, amino acids 63-72 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of PPDNYLYNH (SEQ ID No. 28, amino acids 123-131 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of

WAQAYNTT (SEQ ID No. 29, amino acids 177-186 of IL-4  $\alpha$  receptor), 6 or more consecutive residues of GPASVPTTCCFNLA (SEQ ID No. 30, amino acids 1-14 of eotaxin); 6 or more consecutive residues of FNLANRKIPLQRLES (SEQ ID No. 31, amino acids 11-25 of eotaxin); 6 or more consecutive residues of 5 RITSGKCPQKAVIFKT (SEQ ID No. 32, amino acids 30-43 of eotaxin); and 6 or more consecutive residues of IFKTKLAKDICAD (SEQ ID No. 33, amino acids 40-52 of eotaxin);

genetically fused to a nucleic acid molecule encoding a carrier protein.

#### BRIEF DESCRIPTION OF THE DRAWINGS

10 Fig 1. Inhibition of the binding of human IL-4 to its receptor by mouse sera immunized with a human IL-4 vaccine. Immunized mouse sera were diluted and then incubated with human IL-4. Incubation of the mixed BSA-immunized mouse serum dilutions with IL-4 served as controls. Human IL-4 in the samples was measured using a human IL-4 receptor  $\alpha$  capture-ELISA in which microplates were 15 coated with human IL-4R $\alpha$  which binds to both IL-4 and IL-13.

20 Fig 2. Inhibition of the binding of human IL-13 to its receptor by mouse sera immunized with a human IL-13 vaccine. Immunized mouse sera were diluted and then incubated with human IL-13. Incubation of the mixed BSA-immunized mouse serum dilutions with IL-13 served as controls. Human IL-13 in the samples was measured using a human IL-4 receptor  $\alpha$  capture-ELISA in which microplates were 25 coated with human IL-4R $\alpha$  which binds to both IL-4 and IL-13.

25 Fig 3. Inhibition of cell proliferation by mouse sera immunized with a human IL-13 vaccine. Immunized mouse sera were diluted and then incubated with human IL-13. Incubation of the mixed BSA-immunized mouse serum dilutions with IL-13 served as controls. B9-1-3 cells proliferate upon stimulation of human IL-13. These incubations were cultured with B9-1-3 cells and the cell proliferation was measured using the MTT method.

30 Fig. 4. Identification of the chimeric HBcAg/IL-4 peptide fusion protein using SDS-PAGE and immunoblotting. The chimeric fusion protein containing 1 or 2 human IL-4 peptides (15 amino acid residues, table 1, SEQ ID No. 1) was expressed using E. coli and identified using SDS-PAGE (left) and immunoblotting with rabbit anti-human IL-4 (right). A distinct protein band as indicated by the arrow was presented at the location of the predicted molecular weight in the bacteria

lysate sample of post-induction compared to that of pre-induction. Lane 1: pre-induction; Lane 2: post-induction (A: chimeric HBcAg inserted with 2 peptides); Lane 3: post-induction (B: chimeric HBcAg inserted with 1 peptides).

**Fig. 5.** Identification of the particle behavior of the chimeric HBcAg-IL-4 fusion protein - a comparison of native HBcAg particles with chimeric HBcAg particles (insertion of peptides) by the sedimentation analysis and size exclusion chromatography. (A) Sedimentation analysis. *E. coli* derived protein was sedimented on an analysis sucrose gradient. Thirteen fractions (from the top to the bottom of the centrifugation tube) were analyzed by SDS-PAGE. (B) Size exclusion chromatography using a Sepharose CL-4B column.

**Fig. 6.** Identification of the chimeric HBcAg/IL-4 peptide fusion protein under an electron microscope. Under an electron microscope, the chimeric fusion protein shown in Fig 4. was presented as capsid-like particles with a diameter of 20-30 as indicated by the arrow.

**Fig. 7. Titres of IgG antibodies against human IL-4 measured by an ELISA**  
Mice were immunized 3 times with the chimeric HBcAg containing human IL-4 peptides emulsified in CFA/IFA. Sera were collected 2 weeks after the last immunization.

**Fig. 8. Titres of IgG antibodies against human IL-13 measured by an ELISA.**  
Mice were immunized 3 times with the chimeric HBcAg containing human IL-13 peptide emulsified in CFA/IFA. Sera were collected 2 weeks after the last immunization.

**Fig. 9. Inhibition of cell proliferation by mouse sera immunized with a chimeric HBcAg containing human IL-13 peptides.** Mouse sera were diluted and then incubated with human IL-13. Incubation of the dilutions from a pooled HBcAg-immunized serum with IL-13 served as controls. B9-1-3 cells, which proliferate upon stimulation of human IL-13, were cultured with these incubations. The cell proliferation was measured using the MTT method.

**Fig. 10. The effect of vaccination with an IgE peptide vaccine in downregulation of serum IgE levels in mice and rats.** Mice (A) and rats (B) were vaccinated 3 times with a human IgE peptide-based vaccine (the peptides are up to 92% identical to the mouse IgE peptides and the antibodies induced by the vaccine cross-react to both mouse and rat IgE). In the prevention study, vaccination started 5 weeks before the sensitization commenced and sera were

obtained 1 week after the last sensitization (left panel). In the treatment study, the first vaccination was administered 2 weeks after the last sensitization and sera were collected 2 weeks after the last vaccination (right panel). Sensitized mice/rats receiving injections of the carrier protein served as sham-vaccination controls.

## 5 DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

### DEFINITIONS

As used herein, "effective amount" refers to the administration of an amount of a given compound that achieves the desired effect.

15 As used herein, "purified" does not require absolute purity but is instead intended as a relative definition. For example, purification of starting material or natural material to at least one order of magnitude, preferably two or three orders of magnitude is expressly contemplated as falling within the definition of "purified".

As used herein, the term "isolated" requires that the material be removed  
20 from its original environment.

As used herein, the term "treating" in its various grammatical forms refers to preventing, curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a disease state, disease progression, disease causative agent other abnormal condition.

25 As used herein, "conservative substitution" refers to substitution of an amino acid with an amino acid which has similar properties such that one of skill in the art would anticipate or predict that the secondary structure and hydrophobic nature of the polypeptide would be substantially unchanged.

As used herein, "variant" refers to allotypes known in the art that comprise  
30 one or more amino acid changes within a given sequence.

As used herein, cytokine is preferably a 4 helical cytokine, for example, IL-4, IL-13, IL-5, IL-9, or IL-25; chemokine refers to for example eotaxin and TARC.

The invention comprises cytokine/chemokine, for example, IL-4, IL-13, IL-5,

- IL-9 or IL-25, eotaxin, or TARC-derived peptides or peptides derived from cytokine/chemokine receptors such as IL-4 $\alpha$  receptor-derived peptides linked to a carrier protein and the use thereof as a vaccine for inducing immunity in a patient in need of such treatment. That is, an immunized patient's immune system will
- 5 react to elevated levels of the Th2 cytokines/chemokines by producing antibodies against these cytokine/chemokines, thereby reducing the severity of an allergic or asthmatic response, as discussed below. As discussed below, in most cases, the peptides correspond to helical regions and/or receptor contact sites within the cytokine/chemokine.
- 10 The IL-4 peptides may be selected from the group consisting of: 6 or more consecutive residues of DITLQEIIKTLNSLT (SEQ ID No. 1, amino acids 4-18 of IL-4); 6 or more consecutive residues of EKETFCRAATVLRQFYSHH (SEQ ID No. 2, amino acids 41-59 of IL-4); 6 or more consecutive residues of QQFHRHKQLIRFLKRLDRNLWGLA (SEQ ID No. 3, amino acids 71-94); 6 or more
- 15 consecutive residues of TLENFLERLKTIMREKYS (SEQ ID No. 4, amino acids 108-125); 6 or more consecutive residues of EQKTLCTELTVTDIFA (SEQ ID No. 5, amino acids 19-34); and 6 or more consecutive residues of AGLNSCPVKE (SEQ ID No. 6, amino acids 94-103 of IL-4), as shown in Table 1.

The IL-13 peptides may be selected from the following group: 6 or more consecutive residues of VPPSTALRELIEELVNITQ (SEQ ID No. 7, amino acids 4-22 of IL-13); 6 or more consecutive residues of MYCAALESLI (SEQ ID No. 8, amino acids 43-52 of IL-13); 6 or more consecutive residues of VAQFKDLLLHLKK (SEQ ID No. 9, amino acids 92-105 of IL-13); 6 or more consecutive residues of KDLLLHLKKLFREGFN (SEQ ID No. 10, amino acids 97-25 113 of IL-13); 6 or more consecutive residues of KIEVAQFKDLLLHLKKLFREGFN (SEQ ID No. 11, amino acids 89-113 of IL-13); 6 or more consecutive residues of SAIEKTQRMLSGFC (SEQ ID No. 12, amino acids 58-71 of IL-13); and 6 or more consecutive residues of NVSGCSAIEKTQRMLSGFC (SEQ ID No. 13, amino acids 53-71 of IL-13), as

30 shown in Table 2.

The IL-9 peptides may be selected from the group consisting of: 6 or more consecutive residues of PTLAGILDINF (SEQ ID No. 14, amino acids 4-11 of IL-9); 6 or more consecutive residues of TRYPLIFSRVKKSVE (SEQ ID No. 15, amino

acids 65-79 of IL-9); 6 or more consecutive residues of NALTFLKSLLEI (SEQ ID No. 16, amino acids 102-113 of IL-9); 6 or more consecutive residues of PASKCHCSANVTSCCLCLG (SEQ ID No. 17, amino acids 23-40 of IL-9); 6 or more consecutive residues of CTRPCFSE (SEQ ID No. 18, amino acids 46-53 of IL-9); 5 and 6 or more consecutive residues of KNNKCPYFSCEQPCN (SEQ ID No. 19, amino acids 82-96 of IL-9), as shown in Table 3.

The IL-5 peptide may be selected from the group consisting of: 6 or more consecutive residues of PTSALVKETLALLSTHRTLLIA (SEQ ID No. 20, amino acids 6-27 of IL-5); 6 or more consecutive residues of PTSALVKETLALLST (SEQ 10 ID No. 21, amino acids 6-20 of IL-5); 6 or more consecutive residues of HRTLLIA (SEQ ID No. 22, amino acids 21-27 of IL-5); 6 or more consecutive residues of EERRRVNQFLD (SEQ ID No. 23, amino acids 88-98 of IL-5); 6 or more consecutive residues of TVERLFKNLSLIK (SEQ ID No. 24, amino acids 64-77 of IL-5); and PVHKNH (SEQ ID No. 25, amino acids 36-41 of IL-5), as shown in 15 Table 4.

The IL-4 $\alpha$  receptor peptides may be selected from the group consisting of: 6 or more consecutive residues of LYQLVFLLSEAH (SEQ ID No. 26, amino acids 36-47 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of LLMDDVVSAD (SEQ ID No. 27, amino acids 63-72 of IL-4  $\alpha$  receptor); 6 or more consecutive residues 20 of PPDNYLYNH (SEQ ID No. 28, amino acids 123-131 of IL-4  $\alpha$  receptor); and 6 or more consecutive residues of WAQAYNTT (SEQ ID No. 29, amino acids 177-186 of IL-4  $\alpha$  receptor), as shown in Table 5.

The eotaxin peptide may be selected from the group consisting of 6 or more consecutive residues of GPASVPTTCCFNLA (SEQ ID No. 30, amino acids 1-14 of eotaxin); 6 or more consecutive residues of FNLANRKIPLQRLES (SEQ ID No. 31, amino acids 11-25 of eotaxin); 6 or more consecutive residues of RITSGKCPQKAVIFKT (SEQ ID No. 32, amino acids 30-43 of eotaxin); and 6 or more consecutive residues of IFKTKLAKDICAD (SEQ ID No. 33, amino acids 40-52), as shown in Table 6.

30 As will be apparent to one of skill in the art, other cytokine and/or chemokine derived peptides may also be used, as discussed below.

In other embodiments, the peptide may comprise 7 or more consecutive

residues, 8 or more consecutive residues, 9 or more consecutive residues, or 10 or more consecutive residues of any one of SEQ ID No. 1-33 (where appropriate), or variants thereof. As will be appreciated by one of skill in the art, in embodiments wherein the vaccine is a DNA vaccine or wherein the peptides are produced as 5 genetic fusions co-synthesized with a carrier as discussed below, the nucleic acid sequence may be based on or deduced from any one of the above-described peptides, as described below.

In other embodiments, the peptide may consist essentially of or may consist of 6 or more consecutive residues, 7 or more consecutive residues, 8 or more 10 consecutive residues, 9 or more consecutive residues, or 10 or more consecutive residues of any one of SEQ ID No. 1-33 (where appropriate), or variants thereof. In other embodiments, the peptide may consist essentially of or may consist of any 15 one of SEQ ID No. 1-33 inclusive. As will be appreciated by one of skill in the art, in embodiments wherein the vaccine is a DNA vaccine or wherein the peptides are produced as genetic fusions co-synthesized with a carrier as discussed below, the nucleic acid sequence may be based on or deduced from any one of the above-described peptides, as described below.

As discussed herein, SEQ ID No. 1-33 are human-derived peptide sequences. As will be appreciated by one of skill in the art, the corresponding 20 proteins in other evolutionarily related organisms may have identical or closely related or homologous sequences over the regions corresponding to the peptides designated as SEQ ID No. 1-33. These are also considered to be variants within the scope of the invention. As used herein, evolutionarily related organisms includes for example, but by no means limited to human, rat, mouse and dog.

25 It is of note that it is well known in the art that some modifications and changes can be made in the structure of a polypeptide without substantially altering the biological function of that peptide, to obtain a biologically equivalent polypeptide. In one aspect of the invention, the above-described peptides may include peptides that differ by conservative amino acid substitutions. The peptides 30 of the present invention also extend to biologically equivalent peptides that differ by conservative amino acid substitutions. As used herein, the term "conserved amino acid substitutions" refers to the substitution of one amino acid for another at a given location in the peptide, where the substitution can be made without

substantial loss of the relevant function, in this case, the folding of the epitope. In making such changes, substitutions of like amino acid residues can be made on the basis of relative similarity of side-chain substituents, for example, their size, charge, hydrophobicity, hydrophilicity, and the like, and such substitutions may be assayed for their effect on the function of the peptide by routine testing.

In some embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another having a similar hydrophilicity value (e.g., within a value of plus or minus 2.0), where the following may be an amino acid having a hydropathic index of about -1.6 such as Tyr (-1.3) or Pro (-1.6)s are assigned to amino acid residues (as detailed in United States Patent No. 4,554,101, incorporated herein by reference): Arg (+3.0); Lys (+3.0); Asp (+3.0); Glu (+3.0); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Pro (-0.5); Thr (-0.4); Ala (-0.5); His (-0.5); Cys (-1.0); Met (-1.3); Val (-1.5); Leu (-1.8); Ile (-1.8); Tyr (-2.3); Phe (-2.5); and Trp (-3.4).

In alternative embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another having a similar hydropathic index (e.g., within a value of plus or minus 2.0). In such embodiments, each amino acid residue may be assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics, as follows: Ile (+4.5); Val (+4.2); Len (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (-0.4); Thr (-0.7); Set (-0.8); Trp (-0.9); Tyr (-1.3); Pro (-1.6); His (-3.2); Glu (-3.5); Gln (-3.5); Asp (-3.5); Am (-3.5); Lys (-3.9); and Arg (-4.5).

In alternative embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another in the same class, where the amino acids are divided into non-polar, acidic, basic and neutral classes, as follows: non-polar: Ala, Val, Len, Ile, Phe, Trp, Pró, Met; acidic: Asp, Glu; basic: Lys, Arg, His; neutral: Gly, Ser, Thr, Cys, Asn, Gln, Tyr,

The peptides may be cross-linked to a carrier protein, as discussed below. In other embodiments, nucleic acid molecules deduced from the above-described peptides are prepared and inserted into expression vectors such that the peptides are produced fused to or inserted within suitable carrier proteins, as discussed below. It is also of note that a suitable adjuvant may also be used in combination with the vaccine, as discussed below.

## CONJUGATES

The antigenicity of the peptides may be increased by linking them to a carrier protein. The most commonly used carrier proteins for preparation of a conjugate are bacterial proteins that humans commonly encounter, such as

- 5      Tetanus toxoid and Diphteria toxoid, although other suitable carriers known in the art may also be used. The most common method for preparation of conjugates is glutaraldehyde, SMCC, EDC and NHS methods, although other suitable methods known in the art may also be used. Vaccine compounds prepared in this manner may also be referred to as non-conformation-restrained vaccine compounds.

## 10     FUSIONS

- Fusion proteins have several advantages over conjugates including better quality control, increased antigenicity, and the possibility of combining DNA immunization with protein boosters. Two commonly used protein fusion partners of this type are hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg),<sup>35, 36</sup> although other suitable fusion partners known in the art may also be used. The recombinant HBsAg containing foreign peptides induces a broad but specific immune response to the foreign peptide, because the inserted peptide or polypeptide is natively displayed on the surface of HBsAg or HBcAg particles which improve presentation of the peptide to cells of the immune system. In a preferred embodiment, the plasmid expressing chimeric HBcAg or HBsAg protein is constructed by inserting a cDNA fragment encoding one of the peptides described herein into the polynucleotide sequence corresponding to the immunodominant region of HBcAg or HBsAg. As will be apparent to one of skill in the art, the expression system will include all appropriate control sequences for transcription, translation and replication for use in a given host cell. The fusion protein can be produced in any suitable expression systems or the expression vector can be used for DNA immunization, as described below. It is of note that the carrier proteins discussed above for chemical fusion may also be used for genetic fusions, as may other suitable carrier proteins known in the art.

## 30     VACCINE PREPARATION

The key peptides are selected according to the antigenicity prediction program using the method of Kolaskar and Tongaonkar (1990) and to the three dimensional structure of the target protein reported<sup>37-39</sup> or displayed by the

software "Protein Explore". As will be apparent to one of skill in the art, these methods may be used to identify peptides of interest from other cytokines and/or chemokines, for example, but by no means limited to, IL-25, eotaxin-derived peptides or their receptors. Only those peptides that are likely to elicit strong antibody responses and are located at the receptor binding sites are chosen for the preparation of vaccines.

In some embodiments, the vaccine may be prepared by synthetically synthesizing or otherwise purifying one of the above-described peptides and chemically cross linking the peptide to a suitable carrier using for example the glutaraldehyde method,<sup>40</sup> although any suitable method known in the art which will substantially maintain the native configuration of the peptide may be used.

In other embodiments, the vaccine may be derived from an expression system comprising a nucleic acid molecule deduced from any one of the above-described peptides genetically fused to a suitable carrier protein. In these embodiments, the expression system may be transfected or transformed into a suitable host and the host grown under conditions promoting expression of the fusion protein. The fusion protein may then be purified, for example, column purified based on size or charge or by antibody-binding and prepared for injection into a patient using means known in the art.

In other embodiments, the expression system is prepared as described above and is used directly to immunize a patient, such that the expression system is expressed within transfected cells of the patient, thereby producing antigen within the cells which is in turn recognized by the patient's immune system.

#### ADJUVANT

In some embodiments, the vaccine is administered, for example, co administered with a suitable adjuvant, for example but by no means limited to CpG oligodeoxynucleotides, alum, novasomes or liposomes. It is of note that other suitable adjuvants known in the art may also be used.

Our novel vaccine strategy consists of active immunization with key peptides of IL-4, IL-5, IL-9, IL-13, IL-25 and chemokines as well as their receptors, made immunogenic via linkage to a highly immunogenic carrier protein and an adjuvant. This strategy has the following major advantages over the mAbs or soluble receptors currently in commercial development:

The new active vaccine strategy provides long-term protection with a few injections, while with current strategies, reagents must be injected frequently to maintain their benefits due to a short in vivo half-life.

5 The new vaccine can be used for both prevention and treatment, while current reagents can be used only for treatment.

The invention provides long-term protection with a few injections because the protection relies on host antibodies which are more effective and long lasting than the recombinant mAbs or soluble receptors passively administered. In the current strategies, reagents must be injected frequently to maintain their 10 effectiveness, because these benefits are lost upon discontinuation of treatment due to a short in vivo half-life.

Also, the new vaccine will have no adverse effects induced by intravenous infusion as occurs with humanized mAbs.

There are concerns regarding the consequences of inhibiting Th2 cytokines, 15 because Th2 cytokines and their up-regulated product IgE are thought to be important in the immune defence against parasitic diseases. However, these results are all from rodent studies<sup>41</sup> and are controversial with respect to other studies.<sup>42</sup> Studies of patients with parasite infections reveal an inverse correlation between serum IgE concentration and severity of reinfection or the number of the 20 parasite larvae.<sup>43, 44</sup> IL-4 deficient mice do not demonstrate increased susceptibility to parasitic infections.<sup>45</sup> Most importantly, studies with humanized mAb anti-IgE therapy show that there is no increase in parasitic or other infections.<sup>46-48</sup> Concerning the risk of inducing Th1-mediated autoimmune diseases, it has not been demonstrated that enhancement of a Th2 cytokine response will ameliorate 25 or prevent these disorders.<sup>8</sup> Compared with the CpG (ISS) oligodeoxynucleotide therapy which switches immune responses toward a dominant Th1 response and is currently under development,<sup>49</sup> the proposed strategy has markedly less risk of upregulating autoreactive Th1 responses. In the remote event that the above theoretical risk is real, the risk should be balanced against the benefits of treatment 30 and the very real and often severe side effects of conventional therapies. In addition, genetic polymorphisms of some Th-2 genes such as IL-4 have been associated with asthma severity,<sup>50</sup> making the proposed strategy particularly useful

in the treatment of life-threatening asthma. With precautions, anti-Th2 and -IgE therapies are now recommended to be used in developed countries.<sup>8</sup>

Another concern is long-term blockage of Th2 cytokines. The levels of immune responses induced by such vaccines have been found to be reversible in primate studies<sup>33</sup> being adjusted by the frequency of immunization and the use of various kinds of adjuvants, because the immunogenicity of peptide-based vaccines is weak compared to microbial vaccines.

#### TREATMENT OF DISEASE

As discussed above, asthma is characterized by recurring airway obstruction involving smooth muscle cell proliferation and inflammatory cell infiltration. Specifically, asthma is caused by swelling of bronchial tubes, often as a result of an allergic reaction. This swelling is substantially under the control of histamines and proinflammatory cytokines. Given that vaccination with IL-4, IL-5, IL-9, IL-13, IL-25, eotaxin-derived peptides or their receptors such as the IL-4α receptor-derived peptides fused to a carrier would enable the patient's immune system to produce antibodies to control cytokine production (as discussed above), these substrates would likely lessen the severity of asthma attacks. Thus, an effective amount of the vaccine can be administered to individuals suffering from asthma or individuals at risk of developing asthma and the vaccine would accomplish one or more of the following: decrease the severity of or ameliorate symptoms, decrease the duration of attacks, increase the frequency and duration of remission periods, prevent chronic progression of dyspnea, coughing and wheezing, improve hypoxia, increase forced expiration volume in one second, and improve resistance to airflow and hypocapnia/respiratory alkalosis.

Allergies are the result of overproduction of IgE antibodies to allergens. On first exposure to an allergen, the IgE molecules attach to mast cells. On second exposure, the mast cells induce the symptoms commonly associated with an allergic response. Thus, vaccination of an individual suffering from at least one allergy or at risk of developing at least one allergy with an effective amount of any one of the above-described vaccines will accomplish at least one of the following: decrease severity of symptoms associated with an allergic response, including wheezing, sneezing, runny nose, watery eyes and/or itching; decrease severity of clinical signs, and increase the frequency of allergy-free periods.

In some embodiments, there are provided kits for carrying out the invention. Specifically, the kits may comprise adjuvant(s) and one or more of fusion proteins containing IL-4, IL-5, IL-9, IL-13, IL-25, chemokines and their receptor derived peptides described above. In other embodiments, the kit comprises expression systems comprising a nucleic acid molecule deduced from the amino acid sequence of one or more the above-described peptides genetically fused to or fused within a suitable carrier (DNA vaccines). As discussed above, these expression systems may be used for direct vaccination or may be used to prepare a peptide vaccine for subsequent injection. The protein vaccine can be used alone or in combination with its DNA form. Different vaccines can be used alone or in combination depending upon their effect in down regulation of allergic responses in the patient. For example, IL-4 vaccine can be used alone or in combination with IL-13 or IL-9 vaccine. It is of note that in some embodiments, the kit may include instructions, either in written or electronic form, describing the preparation and/or administration of the vaccine.

The invention will now be described by way of examples. However, the invention is not limited to the examples.

#### EXAMPLE 1 - A human IL-4 vaccine

Preparation of the IL-4 vaccine compound. The vaccine compound was prepared by chemically linking a human IL-4 peptide (15 amino acid residues, table 1. SEQ ID No. 1) to bovine serum albumin (BSA) using the glutaraldehyde method.

Production of mouse anti-human IL-4 serum. Four BABL/c mice were vaccinated with the compound emulsified in CFA adjuvant at the first injection and in IFA adjuvant for the remaining 2 injections, giving a total of three injections with a 3-week interval between injections. Five mice receiving BSA emulsified in the same adjuvants were used as controls. Two weeks after the last injection, blood samples were collected and sera obtained.

Measurement of the titers against the peptide, BSA, and human IL-4. ELISAs were performed. Microplates were coated with the peptide (1 µg/well) or BSA (0.1 µg /well) or human IL-4 (50 ng/well). After washing and blocking with PBS containing 2% gelatine and 0.5% Tween 20, the plates were incubated with test samples and a pooled BSA-immunized mouse serum (2-fold diluted, starting

with 1:500), followed by incubation with an enzyme-conjugated goat anti-mouse IgG. The titer of the test sample was determined with the dilution whose optical density at 410 nm ( $OD_{410}$ ) was equivalent to the  $OD_{410}$  of the control serum at 1:1000. Results are shown below:

5

	Mouse #	Anti-peptide	Anti-BSA	Anti-human IL-4
	1	64,000	320,000	32,000
	2	32,000	320,000	32,000
10	3	128,000	640,000	128,000
	4	64,000	640,000	16,000

ELISA inhibition tests. In order to examine if the mouse sera were able to inhibit the binding of human IL-4 to its receptors, inhibition tests were performed. In the inhibition tests, mouse serum dilutions were 1:25, 1:50, 1:100 and 1:200. Each dilution was mixed with human IL-4 at a final concentration of 50 ng/ml and then incubated for 1 hour at room temperature. Incubation of the dilutions from a pooled BSA-immunized mouse serum with human IL-4 served as a control. Human IL-4 in the mixtures was then measured using a human IL-4 receptor  $\alpha$  (IL-4R $\alpha$ ) capture-ELISA. In the ELISA, the IL-4R $\alpha$  (25 ng/well) was coated on the microplates. After washing and blocking, the plates were incubated with test samples followed by incubations with biotinylated monoclonal anti-human IL-4 and then alkaline phosphatase-conjugated avidin.  $OD_{410}$  was read. The results were expressed using the value of  $OD_{410}$ . The percent of inhibition was calculated as below:

$$OD_{410} \text{ of control sample} - OD_{410} \text{ of test sample}$$

$$\text{Inhibition (\%)} = \frac{OD_{410} \text{ of control samples}}{OD_{410} \text{ of test sample}} \times 100\%$$

As shown in Figure 1, the binding of human IL-4 to its receptor was significantly inhibited in a dose-dependent manner by addition of the mouse anti-human IL-4 serum.

EXAMPLE 2 - A human IL-13 vaccine

Preparation of the IL-13 vaccine compound. The vaccine compound was prepared by chemically linking a human IL-13 peptide (17 amino acid residues, table 2. SEQ ID No. 10) to bovine serum albumin (BSA) using the glutaraldehyde 5 method, described above.

Production of mouse anti-human IL-13 serum. Five BABL/c mice were vaccinated with the human IL-13 compound and CFA/IFA adjuvant while five mice receiving BSA and the same adjuvant served as controls as described in example 1. Two weeks after the last injection, blood samples were collected and sera were 10 obtained.

Measurement of the titers against the peptide, BSA, and human IL-13. ELISAs were performed to measure the titers as described in example 1. In brief, microplates coated with the peptide (1 µg /well) or BSA (0.1 µg /well) or human IL-13 (50 ng/well) were incubated with test samples and a pooled BSA-immunized 15 mouse serum, followed by incubation with an enzyme-conjugated goat anti-mouse IgG. Results are shown below:

Mouse #	Anti-peptide	Anti-BSA	Anti-human IL-13
20			
1	8,000	400,000	16,000
2	2,000	320,000	4,000
3	16,000	640,000	4,000
25	128,000	1,280,000	32,000
5	16,000	1,280,000	2,000

ELISA inhibition tests. In order to determine if the mouse sera were able to 30 inhibit the binding of human IL-13 to its receptors, inhibition tests were performed as described in example 1. In the inhibition tests, mouse serum dilutions were 1:50, 1:100 and 1:200 and were mixed with human IL-13 at a final concentration of 200 ng/ml and then incubated for 1 hour at room temperature. Incubation of

dilutions of a pooled BSA-immunized mouse serum with human IL-13 served as controls. Human IL-13 in the mixtures was then measured using a human IL-4 receptor  $\alpha$  (IL-4R $\alpha$ ) capture-ELISA as described in example 1, because both human IL-4 and IL-13 bind to IL-4R $\alpha$ . The binding of human IL-13 to its receptor  
5 was significantly inhibited in a dose-dependent manner by addition of the mouse anti-human IL-13 serum (**Figure 2**).

Cell binding inhibition tests. B9-1-3 cells, which proliferate upon stimulation of human IL-13,<sup>51</sup> were used to determine if the vaccinated mouse sera could inhibit the binding of human IL-13 to its receptors on the cells. Mouse sera were  
10 diluted (final dilution 1:20 and 1:40) and then incubated with human IL-13 (final concentration 10 ng/ml). Incubation of the pooled BSA-immunized mouse serum dilutions with IL-13 served as controls. These incubations were cultured with B9-1-3 cells and the cell proliferation was measured using the MTT method.<sup>52</sup> The results were expressed by measuring optical density at 570 nm. The binding of  
15 human IL-13 to its receptors on the cells as measured by proliferation of cells was significantly inhibited in a dose-dependent manner by addition of the mouse anti-human IL-13 serum (**Figure 3**).

EXAMPLE 3 – A recombinant human IL-4 vaccine: HBcAg-IL-4 capsid-like fusion protein

20 Construction of recombinant plasmid. The polynucleotides encoding for Hepatitis B virus core antigen were cloned into plasmid pThioHisA (Invitrogen, Inc.) between the restriction enzyme sites for Nde I and Pst I such that the insert was under the control of a strong promoter P<sub>trc</sub>, resulting a new recombinant plasmid which expresses HBV core antigen.

25 The chimeric protein expression vector was constructed by introducing a new Kpn I site by PCR mutagenesis at nucleotide position between 235 and 237 of the above HBV core antigen, which allows the insertion of exogenous epitopes. The oligonucleotide encoding for the human IL-4 key antigenic peptide (Table 1. SEQ ID No. 1) was synthesized. Positive and negative chain oligonucleotides were  
30 mixed and denatured at 95°C for 5 min, and then denatured by gradually reducing the temperature to room temperature. The segment and the vector digested with Kpn I were ligated at 16°C overnight and transformed into DH5a competent cells.

The recombinants with the correct insertion orientation were identified by restriction endonuclease digestion and PCR.

Expression of the recombinant HBcAg-IL-4 protein. A single colony was picked and added to 2 ml of fresh LB medium and cultured at 37°C overnight. The 5 culture was transferred into fresh LB at 3-5% inoculation volume, and incubated at 37°C with vigorous shaking. When the OD<sub>600</sub> reached 0.6-0.8, IPTG was added to a final concentration of 1 mmol/L to induce the expression of recombinant proteins. After 4-6 hours, the bacteria were harvested by centrifuging at 2,000 g and the pellets were re-suspended with 20 mmol/L phosphate saline buffer (pH 7.4), which contains 10 150 mmol/L NaCl and 10 mmol/L EDTA. The bacteria were sonicated, and after centrifuging, the supernatants were subjected to sucrose gradient centrifuging.

SDS-PAGE and immunoblotting. SDS-PAGE and immunoblot analysis was performed to identify the IL-4 antigenicity of the HBcAg-IL-4 fusion protein. The primary antibody was the rabbit anti-human IL-4 antibody (Peprotech Canada, Inc.), while the secondary antibody was alkaline phosphatase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc.). As shown in Figure 15 4, the rabbit anti-human IL-4 recognizes the fusion protein.

Identification of its capsid-like particle properties. Sedimentation analysis using sucrose gradient centrifuging, size exclusion chromatography, and electron 20 microscopy were used to determine if the fusion protein was present as capsid-like particles. The sucrose step gradient was prepared by sequentially loading 1.9 ml of 60%, 50%, 40%, 30%, 20% and 0.5 ml of 10% sucrose solution into a 12 ml ultracentrifuge tube. Two millilitres of the supernatant sample of bacteria lysates were loaded onto the top of the gradient. The recombinant plasmid expressing 25 HBV core antigens, which are known to form particles, served as a control. Centrifuging was conducted for 3 hr at 36,000 rpm at 20°C. Twelve 1 ml-fractions were collected from the top to the bottom of the gradient. The 12 fractions were analysed by SDS-PAGE (Figure 5 A) and the fraction containing capsid-like particles (fractions 6) was loaded onto a Sepharose CL-4B column (Figure 5 B), 30 and also examined by electron microscopy (Figure 6). As shown in Figures 5 and 6, the fusion protein is presented as capsid-like particles with a diameter of 20-30 nm, which are highly immunogenic.

Production of mouse anti-human IL-4 serum. Four BABL/c mice were vaccinated with the chimeric HBcAg-IL-4 fusion protein emulsified in CFA adjuvant at the first injection and in IFA adjuvant for the remaining 2 injections giving a total of three injections with a 3-week interval between 2 injections. Four mice receiving 5 the recombinant HBcAg emulsified in the same adjuvant served as controls. Two weeks after the last injection, blood samples were collected and sera were obtained.

Measurement of the titers against human IL-4. ELISAs were performed to determine the titre to human IL-4. In brief, microplates were coated with human IL-10 4 (50 ng/well). After washing and blocking with PBS containing 2% gelatine and 0.5% Tween 20, the plates were incubated with test samples and a pooled recombinant HBcAg-immunized control serum (2-fold diluted, starting with 1:500), followed by incubation with an enzyme-conjugated goat anti-mouse IgG. The titer of the test sample was determined at the dilution whose optical density at 410 nm 15 (OD<sub>410</sub>) was at least 2.1 fold of the OD<sub>410</sub> of the control serum at the same dilution when its value was around 0.1. The titres to human IL-4 were between 1:128,000 and 256,000 (**Figure 7**)

EXAMPLE 4 – A recombinant human IL-4 vaccine: HBcAg-IL-13 capsid-like fusion protein

20 The recombinant HBcAg-IL-13 capsid-like fusion protein was constructed, expressed, and identified as described in example 3. The inserted peptide is derived from human IL-13 receptor binding sides SEQ ID No. 7. SDS-PAGE and immunoblot analysis with a rabbit anti-human IL-13 antibody (Peprotech Canada, Inc.) and an alkaline phosphatase-conjugated goat anti-rabbit IgG (Jackson

25 ImmunoResearch Laboratories, Inc.) showed that the rabbit anti-human IL-13 recognizes the fusion protein. Sedimentation analysis using sucrose gradient centrifuging and size exclusion chromatography with Sepharose CL-4B showed that the recombinant HBcAg-IL-13 fusion protein was presented as virus-like particles.

30 Four BABL/c mice were vaccinated with the recombinant HBcAg-IL-13 fusion protein while four mice receiving recombinant HBcAg served as controls as described in example 1. Blood samples were collected and sera were tested for the titres to human IL-13 using an ELISA as described in example 3. In the ELISA,

microplates coated with human IL-13 (50 ng/well) were incubated with test samples and a pooled HBcAg-immunized mouse serum, followed by incubation with an enzyme-conjugated goat anti-mouse IgG. The titres to human IL-4 were between 1:128,000 and 256,000 (**Figure 8**).

5 Cell proliferation inhibition tests were performed using B9-1-3 cells as described in example 2 to determine if the vaccinated mouse sera can inhibit the binding of human IL-13 to its receptors. The results showed that the binding of human IL-13 to its receptors on the cells, as measured by cell proliferation, was significantly inhibited in a dose-dependent manner by addition of the mouse anti-  
10 human IL-13 serum (**Figure 9**).

**EXAMPLE 5 – Concept proof of peptide-based vaccinations in downregulation of serum IgE responses in mice and rats.**

In order to demonstrate the effect of peptide-based vaccines in the down regulation of allergic responses *in vivo*, we vaccinated mice and rats with a human IgE peptide-based vaccine which induces neutralizing antibodies blocking the binding of human IgE to its receptors *in vitro* as measured by a human Fc $\epsilon$ RI-capture ELISA and flow cytometric analysis. Because the peptides derived from the human IgE receptor binding site are up to 92% identical to the corresponding murine IgE peptides, and the antiserum raised by the vaccine bound not only to human IgE but also to mouse and rat IgE, *in vivo* rat and mouse experiments were performed as below:

25 Preparation of IgE vaccine compound. The vaccine compound was prepared by chemically linking 3 human IgE peptides (8, 12, and 16 amino acid residuals, respectively) to the carrier protein, hepatitis B surface antigen (HBsAg), using the glutaraldehyde method.

30 Vaccination in the prevention and treatment studies. Groups of 4 mice (A) and 4 rats (B) were sensitized with TKM allergen mixed with adjuvant alum twice at an interval of 3 weeks. An IgE vaccine was administered subcutaneously 3 times at an interval of 2 - 3 weeks. In the prevention study, the vaccination started 5 weeks before sensitization and serum samples were obtained 1 week after the last sensitization (left panel). In the treatment study, the first vaccination was administered 2 weeks after the last sensitization and the serum samples were

collected 2 weeks after the last vaccination (right panel). Mice and rats receiving injections of the carrier protein (HBsAg) served as controls. Serum free IgE (not bound to anti-IgE antibodies) was measured by passive cutaneous anaphylaxis (PCA) tests.

5        PCA tests. Mouse and rat sera were diluted and intradermally injected into a normal recipient rat skin in duplicate. TKM-allergic mouse serum dilutions served as positive controls and saline served as negative controls. Twenty four hours later, Evans Blue mixed with TKM allergen was intravenously injected. The skin was cut and the Evans Blue in the skin was dissolved in an acetone saline solution. The colour of the solution was measured at OD<sub>610</sub> nm. Inhibition was calculated as described previously.

10      Results. Vaccination of rats and mice with an IgE vaccine significantly inhibited subsequent increases in serum IgE levels (Fig 10, left panels), while vaccination of sensitized rats and mice reduced the elevated serum IgE levels 15 upon allergen re-exposure (Fig 10, right panels). Because serum specific IgE levels are correlated with airway hyperresponsiveness,<sup>21-23</sup> these proof of concept studies show promise for treatment of asthmatic individuals with Th2 cytokine/chemokine peptide-based vaccines.

15      While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.

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15

**Table 1.** Core sequences of human IL-4 vaccines<sup>1-4</sup>

ID No	Start position	Sequence	End position	No. of aa	Location
1	4	DIT <u>LQEI</u> <b>KT</b> LNSLT	18	15	Helix A
2	41	EKETFCRAATVLRQFYSHH	59	19	Helix B
3	71	QQFHRHKQL <u>I</u> RFL <u>K</u> RLD <u>R</u> NLW GLA	94	24	Helix C
4	108	TLENFLERLK <u>T</u> IM <u>R</u> E <u>K</u> YS	125	18	Helix D
5*	19	EQKTLCTELTVTDIFA	34	16	not in the $\alpha$ contact sites
6*	94	AGLNSCP <span style="color:red">V</span> K <span style="color:red">E</span>	103	10	not in the $\alpha$ contact sites

Bold and underlined represent those amino acids that come directly into contact with the IL-4R $\alpha$  cited by both Eisenmesser EZ et al.<sup>2</sup> and Moy FJ et al.<sup>1</sup>

Bold, italic and underline represent those amino acids that come directly into contact with the IL-4R $\alpha$  cited by Moy FJ et al.<sup>1</sup>

Red and bold represented those amino acids cited by Hage T et al.<sup>3</sup>

**Table 2.** Core sequences of human IL-13 vaccines<sup>1-4</sup>

ID No	Start position	Sequence	End position	No. of aa	Location
7	4	VPPSTALRELIEELVNITQ	22	19	Helix A
8	43	MYCAALESLI	52	10	Helix B
9	92	VAQFVKDLLHL <u>KK</u>	105	14	Helix D
10	97	KDLLLHL <u>KKLF</u> <b>R</b> EGRFN	113	17	Helix D
11	89	KIEVAQFV KDLLLHL <u>KKLF</u> <b>R</b> EGRFN	113	25	Helix D
12	58	SAIE <u>KTQRMLSGFC</u>	71	14	Helix C
13	53	NVSGCSAIE <u>KTQRMLSGF</u> C	71	19	Helix C

Bold represents those amino acids that come into direct contact with the corresponding receptor.

**Table 3.** Core sequences of human IL-9 vaccines<sup>5</sup>

ID No.	Start position	Sequence	End position	No. of aa	Location
14	4	PTLAGILDINF	14	11	Helix A
15	65	TRYPLIFSRVKKSVE	79	15	Helix C
16	102	NALTFLKSLLEI	113	12	Helix D
17*	23	PASKCHCSANVTSCCLCG	40	18	containing glycosylation sites and disulfate bonds
18*	46	CTRPFSE	53	8	containing glycosylation sites and disulfate bonds
19*	82	KNNKCPYFSCEQPCN	96	15	containing glycosylation sites and disulfate bonds

\* Low priority due to containing glycosylation sites and disulfate bonds

**Table 4.** Core sequences of human IL-5 vaccines<sup>6,7</sup>

ID No.	Start position	Sequence	End position	No. of aa	Location
20	6	PTSALV <u>KET</u> LALLST <u>HRT</u> LLIA	27	22	Helix A (optional)
21	6	PTSALV <u>KET</u> LALLST	20	15	Helix A
22	21	<u>HRT</u> LLIA	27	7	Helix A
23	88	E <u>ERRRVNQF</u> LD	98	11	Helix D
24	64	TVERLFKN <u>LSL</u> IKK	77	14	Helix C, not in contact sites (optional)
25	36	PVHKNH	41	6	Neither helix region nor contact site (optional)

Bold represents those amino acids that come into direct contact with the corresponding receptor.

**Table 5.** Core sequences of human IL-4 alpha-receptor vaccines which will prevent the binding of both IL-4 and IL-13 to the alpha receptor.

ID No.	Start position	Sequence	End position	No. of aa	Location
26	36	LYQLVFLLSEAH	47	12	Loop 2
27	63	LLMDDVVSAD	72	10	Loop 3
28	123	PPDNYLYNH	131	9	Loop 5
29	177	WAQAYNTT	186	8	Loop 6

**Table 6.** Core sequences of human eotaxin vaccines<sup>8-10</sup>

ID No.	Start position	Sequence	End position	No. of aa	Location
30	1	<i>GPASVPTTCCFNLA</i>	14	14	flexible N terminal region
31	11	<i>FNLANR<b>KIPLQRLES</b></i>	25	15	N-loop,3 <sub>10</sub> -helical turn
32	30	<i>R/ITSGKCPQKAVIFKT</i>	43	14	30-s loop, β2-strand
33	40	<i>IFKTKLAKDICAD</i>	52	13	β3-strand

Italic letters present the extended amino acid residues not included in the predicted antigen epitope.

Bold and underlined represent those amino acids that play important roles in binding to and activation of the α receptor CCR3 cited by Mayer MR, et al.<sup>9</sup>

The binding surface of eotaxin appears to consist of an extended groove whose borders are defined by residues from the N-loop,310-helical turn, and β2-β3 hairpin regions.<sup>10</sup>

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CLAIMS

1. A reagent comprising:
  - a cytokine or chemokine-derived peptide or a cytokine-receptor-derived peptide or chemokine-receptor derived-peptide;
  - 5 a carrier protein; and
  - an adjuvant.
2. The reagent according to claim 1 wherein the cytokine or chemokine is selected from the group consisting of IL-4, IL-13, IL-5, IL-9, IL-25, eotaxin or  
10 TARC 3.
3. The reagent according to claim 1 wherein the cytokine is IL-4 and the peptide is selected from the group consisting of: 6 or more consecutive residues of DITLQEIIKTLNSLT (SEQ ID No. 1, amino acids 4-18 of IL-4); 6 or more consecutive residues of EKETFCRAATVLRQFYSHH (SEQ ID No. 2, amino acids  
15 41-59 of IL-4); 6 or more consecutive residues of QQFHRHKQLIRFLKRLDRNLWGLA (SEQ ID No. 3, amino acids 71-94 of IL-4); 6 or more consecutive residues of TLENFLERLKTIMREKYS (SEQ ID No. 4, amino acids 108-125 of IL-4); 6 or more consecutive residues of EQKTLCTELTVTDIFA (SEQ ID No. 5, amino acids 19-34 of IL-4); and 6 or more consecutive residues of  
20 AGLNSCPVKE (SEQ ID No. 6, amino acids 94-103 of IL-4).
4. The reagent according to claim 1 wherein the cytokine is IL-13 and the peptide is selected from the group consisting of 6 or more consecutive residues of VPPSTALRELIEELVNITQ (SEQ ID No. 7, amino acids 4-22 of IL-13); 6 or more consecutive residues of MYCAALES LI (SEQ ID No. 8, amino acids 43-  
25 52 of IL-13); 6 or more consecutive residues of VAQFVKDLLLHLKK (SEQ ID No. 9, amino acids 92-105 of IL-13); 6 or more consecutive residues of KDLLLHLKKLFREGRFN (SEQ ID No. 10, amino acids 97-113 of IL-13); 6 or more consecutive residues of KIEVAQFVKDLLLHLKKLFREGRFN (SEQ ID No. 11, amino acids 89-113 of IL-13); 6 or more consecutive residues of  
30 SAIEKTQRMLSGFC (SEQ ID No. 12, amino acids 58-71 of IL-13); and 6 or more consecutive residues of NVSGCSAIEKTQRMLSGFC (SEQ ID No. 13, amino acids 53-71 of IL-13).
5. The reagent according to claim 1 wherein the cytokine is IL-9 and the

- peptide is selected from the group consisting of 6 or more consecutive residues of PTLAGILDINF (SEQ ID No. 14, amino acids 4-11 of IL-9); 6 or more consecutive residues of TRYPLIFSRVKKSV (SEQ ID No. 15, amino acids 65-79 of IL-9); 6 or more consecutive residues of NALTFLKSLLEI (SEQ ID No. 16, amino acids 102-113 of IL-9); 6 or more consecutive residues of PASKCHCSANVTSCCLLG (SEQ ID No. 17, amino acids 23-40 of IL-9); 6 or more consecutive residues of CTRPCFSE (SEQ ID No. 18, amino acids 46-53 of IL-9); and 6 or more consecutive residues of KNNKCPYFSCEQPCN (SEQ ID No. 19, amino acids 82-96 of IL-9).
10. 6. The reagent according to claim 1 wherein the cytokine is IL-5 and the peptide is selected from the group consisting of 6 or more consecutive residues of PTSALVKETLALLSTHRTLLIA (SEQ ID No. 20, amino acids 6-27 of IL-5); 6 or more consecutive residues of PTSALVKETLALLST (SEQ ID No. 21, amino acids 6-20 of IL-5); 6 or more consecutive residues of HRTLLIA (SEQ ID No. 22, amino acids 21-27 of IL-5); 6 or more consecutive residues of EERRRVNQFLD (SEQ ID No. 23, amino acids 88-98 of IL-5); 6 or more consecutive residues of TVERLFKNLSLIK (SEQ ID No. 24, amino acids 64-77 of IL-5); and PVHKNH (SEQ ID No. 25, amino acids 36-41 of IL-5).
7. The reagent according to claim 1 wherein the cytokine receptor is IL-4  $\alpha$  receptor and the peptide is selected from the group consisting of 6 or more consecutive residues of LYQLVFLLSEAH (SEQ ID No. 26, amino acids 36-47 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of LLMDDVVSAD (SEQ ID No. 27, amino acids 63-72 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of PPDNYLYNH (SEQ ID No. 28, amino acids 123-131 of IL-4  $\alpha$  receptor); and 6 or more consecutive residues of WAQAYNTT (SEQ ID No. 29, amino acids 177-186 of IL-4  $\alpha$  receptor).
8. The reagent according to claim 1 wherein the adjuvant is selected from the group consisting of: CpG oligodeoxynucleotides, alum, novasomes and liposomes.
30. 9. The reagent according to claim 1 wherein the carrier protein is a highly immunogenic carrier protein.
10. The reagent according to claim 9 wherein the carrier protein is

selected from the group consisting of hepatitis B core antigen and hepatitis B surface antigen.

11. The reagent according to claim 10 wherein the peptide is fused into an immunodominant region of the carrier protein.

5 12. A method of inducing an immune response in an individual, comprising:

administering to an individual in need of such a treatment, an effective amount of a composition comprising:

a cytokine-derived peptide or chemokine-derived peptide or a cytokine-  
10 receptor derived peptide or chemokine-receptor derived-peptide;  
a carrier protein; and  
an adjuvant.

13. The method according to claim 12 wherein the cytokine is selected from the group consisting of IL-4, IL-13, IL-5, IL-9 or IL-25.

15 14. The method according to claim 12 wherein the cytokine is IL-4 and the peptide is selected from the group consisting of: 6 or more consecutive residues of DITLQEIIKTLNSLT (SEQ ID No. 1, amino acids 4-18 of IL-4); 6 or more consecutive residues of EKETFCRAATVLRQFYSHH (SEQ ID No. 2, amino acids 41-59 of IL-4); 6 or more consecutive residues of 20 QQFHRHKQLIRFLKRLDRNLWGLA (SEQ ID No. 3, amino acids 71-94 of IL-4); 6 or more consecutive residues of TLENFLERLKTIMREKYS (SEQ ID No. 4, amino acids 108-125 of IL-4); 6 or more consecutive residues of EQKTLCTELTVTDIFA (SEQ ID No. 5, amino acids 19-34 of IL-4); and 6 or more consecutive residues of AGLNSCPVKE (SEQ ID No. 6, amino acids 94-103 of IL-4).

25 15. The method according to claim 12 wherein the cytokine is IL-13 and the peptide is selected from the group consisting of 6 or more consecutive residues of VPPSTALRELIEELVNITQ (SEQ ID No. 7, amino acids 4-22 of IL-13); 6 or more consecutive residues of MYCAALESI (SEQ ID No. 8, amino acids 43-52 of IL-13); 6 or more consecutive residues of VAQFVKDLLHLKK (SEQ ID No. 30 9, amino acids 92-105 of IL-13); 6 or more consecutive residues of KDLLLHLKKLFREGRFN (SEQ ID No. 10, amino acids 97-113 of IL-13); 6 or more consecutive residues of KIEVAQFVKDLLHLKKLFREGRFN (SEQ ID No. 11, amino acids 89-113 of IL-13); 6 or more consecutive residues of

SAIEKTQRMLSGFC (SEQ ID No. 12, amino acids 58-71 of IL-13); and 6 or more consecutive residues of NVSGCSAIEKTQRMLSGFC (SEQ ID No. 13, amino acids 53-71 of IL-13).

16. The method according to claim 12 wherein the cytokine is IL-9 and  
5 the peptide is selected from the group consisting of 6 or more consecutive residues of PTLAGILDINF (SEQ ID No. 14, amino acids 4-11 of IL-9); 6 or more consecutive residues of TRYPLIFSRVKKSV (SEQ ID No. 15, amino acids 65-79 of IL-9); 6 or more consecutive residues of NALTFLKSLLEI (SEQ ID No. 16, amino acids 102-113 of IL-9); 6 or more consecutive residues of  
10 PASKCHCSANVTSCCLLG (SEQ ID No. 17, amino acids 23-40 of IL-9); 6 or more consecutive residues of CTRPCFSE (SEQ ID No. 18, amino acids 46-53 of IL-9); and 6 or more consecutive residues of KNNKCPYFSCEQPCN (SEQ ID No. 19, amino acids 82-96 of IL-9).

17. The method according to claim 12 wherein the cytokine is IL-5 and  
15 the peptide is selected from the group consisting of 6 or more consecutive residues of PTSALVKETLALLSTHRTLLIA (SEQ ID No. 20, amino acids 6-27 of IL-5); 6 or more consecutive residues of PTSALVKETLALLST (SEQ ID No. 21, amino acids 6-20 of IL-5); 6 or more consecutive residues of HRTLLIA (SEQ ID No. 22, amino acids 21-27 of IL-5); 6 or more consecutive residues of EERRRVNQFLD  
20 (SEQ ID No. 23, amino acids 88-98 of IL-5); 6 or more consecutive residues of TVERLFKNLSLIK (SEQ ID No. 24, amino acids 64-77 of IL-5); and PVHKNH (SEQ ID No. 25, amino acids 36-41 of IL-5).

18. The method according to claim 12 wherein the cytokine receptor is IL-4  $\alpha$  receptor and the peptide is selected from the group consisting of 6 or more consecutive residues of LYQLVFLLSEAH (SEQ ID No. 26, amino acids 36-47 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of LLMDDVVSAD (SEQ ID No. 27, amino acids 63-72 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of PPDNYLYNH (SEQ ID No. 28, amino acids 123-131 of IL-4  $\alpha$  receptor); and 6 or more consecutive residues of WAQAYNTT (SEQ ID No. 29, amino acids 177-186  
30 of IL-4  $\alpha$  receptor).

19. The method according to claim 12 wherein the composition includes an adjuvant.

20. The method according to claim 19 wherein the adjuvant is selected from the group consisting of: CpG oligodeoxynucleotides, alum, novasomes and liposomes.

21. The method according to claim 12 wherein the carrier protein is a  
5 highly immunogenic carrier protein.

22. The method according to claim 21 wherein the carrier protein is selected from the group consisting of hepatitis B core antigen and hepatitis B surface antigen.

23. The method according to claim 22 wherein the peptide is fused into  
10 an immunodominant region of the carrier protein.

24. A method of treating, ameliorating or preventing asthma comprising:  
administering to an individual in need of such a treatment, an effective amount of a  
composition comprising:

a cytokine-derived peptide or a chemokine-derived peptide or a cytokine-  
15 receptor derived peptide or chemokine-receptor derived-peptide;  
a carrier protein; and  
an adjuvant.

25. The method according to claim 24 wherein the cytokine is selected  
from the group consisting of IL-4, IL-13, IL-2, IL-3, IL-5, IL-9 or IL-25.

26. The method according to claim 24 wherein the cytokine is IL-4 and  
the peptide is selected from the group consisting of: 6 or more consecutive  
residues of DITLQEIIKTLNSLT (SEQ ID No. 1, amino acids 4-18 of IL-4); 6 or  
more consecutive residues of EKETFCRAATVLRQFYSHH (SEQ ID No. 2, amino  
acids 41-59 of IL-4); 6 or more consecutive residues of  
25 QQFHRHKQLIRFLKRLDRNLWGLA (SEQ ID No. 3, amino acids 71-94 of IL-4); 6  
or more consecutive residues of TLENFLERLKTIIMREKYS (SEQ ID No. 4, amino  
acids 108-125 of IL-4); 6 or more consecutive residues of EQKTLCTELTVTDIFA  
(SEQ ID No. 5, amino acids 19-34 of IL-4); and 6 or more consecutive residues of  
AGLNSCPVKE (SEQ ID No. 6, amino acids 94-103 of IL-4).

30 27. The method according to claim 24 wherein the cytokine is IL-13 and  
the peptide is selected from the group consisting of 6 or more consecutive  
residues of VPPSTALRELIEELVNITQ (SEQ ID No. 7, amino acids 4-22 of IL-13);  
6 or more consecutive residues of MYCAALESLI (SEQ ID No. 8, amino acids 43-

52 of IL-13); 6 or more consecutive residues of VAQFVKDLLLHLKK (SEQ ID No. 9, amino acids 92-105 of IL-13); 6 or more consecutive residues of KDLLLHLKKLFREGRFN (SEQ ID No. 10, amino acids 97-113 of IL-13); 6 or more consecutive residues of KIEVAQFVKDLLLHLKKLFREGRFN (SEQ ID No. 11, 5 amino acids 89-113 of IL-13); 6 or more consecutive residues of SAIEKTQRMLSGFC (SEQ ID No. 12, amino acids 58-71 of IL-13); and 6 or more consecutive residues of NVSGCSAIEKTQRMLSGFC (SEQ ID No. 13, amino acids 53-71 of IL-13).

28. The method according to claim 24 wherein the cytokine is IL-9 and 10 the peptide is selected from the group consisting of 6 or more consecutive residues of PTLAGILDINF (SEQ ID No. 14, amino acids 4-11 of IL-9); 6 or more consecutive residues of TRYPLIFSRVKKSV (SEQ ID No. 15, amino acids 65-79 of IL-9); 6 or more consecutive residues of NALTFLKSLLEI (SEQ ID No. 16, amino acids 102-113 of IL-9); 6 or more consecutive residues of 15 PASKCHCSANVTSCCLCG (SEQ ID No. 17, amino acids 23-40 of IL-9); 6 or more consecutive residues of CTRPCFSE (SEQ ID No. 18, amino acids 46-53 of IL-9); and 6 or more consecutive residues of KNNKCPYFSCEQPCN (SEQ ID No. 19, amino acids 82-96 of IL-9).

29. The method according to claim 24 wherein the cytokine is IL-5 and 20 the peptide is selected from the group consisting of 6 or more consecutive residues of PTSALVKETLALLSTHRTLLIA (SEQ ID No. 20, amino acids 6-27 of IL-5); 6 or more consecutive residues of PTSALVKETLALLST (SEQ ID No. 21, amino acids 6-20 of IL-5); 6 or more consecutive residues of HRTLLIA (SEQ ID No. 22, amino acids 21-27 of IL-5); 6 or more consecutive residues of EERRRVNQFLD 25 (SEQ ID No. 23, amino acids 88-98 of IL-5); 6 or more consecutive residues of TVERLFKNLSLIK (SEQ ID No. 24, amino acids 64-77 of IL-5); and PVHKNH (SEQ ID No. 25, amino acids 36-41 of IL-5).

30. The method according to claim 24 wherein the cytokine receptor is IL-4  $\alpha$  receptor and the peptide is selected from the group consisting of 6 or more 30 consecutive residues of LYQLVFLLSEAH (SEQ ID No. 26, amino acids 36-47 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of LLMDDVVSAD (SEQ ID No. 27, amino acids 63-72 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of

PPDNYLYNH (SEQ ID No. 28, amino acids 123-131 of IL-4  $\alpha$  receptor); and 6 or more consecutive residues of WAQAYNTT (SEQ ID No. 29, amino acids 177-186 of IL-4  $\alpha$  receptor).

31. The method according to claim 24 wherein the composition includes  
5 an adjuvant.

32. The method according to claim 31 wherein the adjuvant is selected from the group consisting of: CpG oligodeoxynucleotides, alum, novasomes and liposomes.

33. The method according to claim 24 wherein the carrier protein is a  
10 highly immunogenic carrier protein.

34. The method according to claim 33 wherein the carrier protein is selected from the group consisting of hepatitis B core antigen and hepatitis B surface antigen.

35. The method according to claim 24 wherein the peptide is fused into  
15 an immunodominant region of the carrier protein.

36. An expression system comprising:  
a nucleic acid molecule deduced from a peptide selected from the group consisting of: 6 or more consecutive residues of DITLQEIIKTLNSLT (SEQ ID No. 1, amino acids 4-18 of IL-4); 6 or more consecutive residues of EKETFCRAATVLRQFYSHH (SEQ ID No. 2, amino acids 41-59 of IL-4); 6 or more consecutive residues of QQFHRHKQLIRFLKRLDRNLWGLA (SEQ ID No. 3, amino acids 71-94); 6 or more consecutive residues of TLENFLERLKTIMREKYS (SEQ ID No. 4, amino acids 108-125); 6 or more consecutive residues of EQKTLCTELTVTDIFA (SEQ ID No. 5, amino acids 19-34); 6 or more consecutive residues of AGLNSCPVKE (SEQ ID No. 6, amino acids 94-103 of IL-4); 6 or more consecutive residues of VPPSTALRELIEELVNITQ (SEQ ID No. 7, amino acids 4-22 of IL-13); 6 or more consecutive residues of MYCAALESLI (SEQ ID No. 8, amino acids 43-52 of IL-13); 6 or more consecutive residues of VAQFVKDLLLHLKK (SEQ ID No. 9, amino acids 92-105 of IL-13); 6 or more consecutive residues of KDLLLHLKKLFREGRFN (SEQ ID No. 10, amino acids 97-113 of IL-13); 6 or more consecutive residues of KIEVAQFVKDLLLHLKKLFREGRFN (SEQ ID No. 11, amino acids 89-113 of IL-13); 6 or more consecutive residues of SAIEKTQRMLSGFC (SEQ ID No. 12,

amino acids 58-71 of IL-13); 6 or more consecutive residues of NVSGCSAIEKTQRMLSGFC (SEQ ID No. 13, amino acids 53-71 of IL-13); 6 or more consecutive residues of PTLAGILDINF (SEQ ID No. 14, amino acids 4-11 of IL-9); 6 or more consecutive residues of TRYPLIFSRVKKSV (SEQ ID No. 15, 5 amino acids 65-79 of IL-9); 6 or more consecutive residues of NALTFLKSLLEI (SEQ ID No. 16, amino acids 102-113 of IL-9); 6 or more consecutive residues of PASKCHCSANVTCLCLG (SEQ ID No. 17, amino acids 23-40 of IL-9); 6 or more consecutive residues of CTRPCFSE (SEQ ID No. 18, amino acids 46-53 of IL-9); 6 or more consecutive residues of KNNKCPYFSCEQPCN (SEQ ID No. 19, amino 10 acids 82-96 of IL-9); 6 or more consecutive residues of PTSALVKETLALLSTHRTLLIA (SEQ ID No. 20, amino acids 6-27 of IL-5); 6 or more consecutive residues of PTSALVKETLALLST (SEQ ID No. 21, amino acids 6-20 of IL-5); 6 or more consecutive residues of HRTLLIA (SEQ ID No. 22, amino acids 21-27 of IL-5); 6 or more consecutive residues of EERRRVNQFLD (SEQ ID 15 No. 23, amino acids 88-98 of IL-5); 6 or more consecutive residues of TVERLFKNLSLIK (SEQ ID No. 24, amino acids 64-77 of IL-5); PVHKNH (SEQ ID No. 25, amino acids 36-41 of IL-5); 6 or more consecutive residues of LYQLVFLLSEAH (SEQ ID No. 26, amino acids 36-47 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of LLMDDVVSAD (SEQ ID No. 27, amino acids 63-72 20 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of PPDNYLYNH (SEQ ID No. 28, amino acids 123-131 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of WAQAYNTT (SEQ ID No. 29, amino acids 177-186 of IL-4  $\alpha$  receptor); 6 or more consecutive residues of GPASVPTTCCFNLA (SEQ ID No. 30, amino acids 1-14 of eotaxin); 6 or more consecutive residues of FNLANRKIPLQRLES (SEQ ID No. 31, 25 amino acids 11-25 of eotaxin); 6 or more consecutive residues of RITSGKCPQKAVIFKT (SEQ ID No. 32, amino acids 30-43 of eotaxin); and 6 or more consecutive residues of IFKTKLAKDICAD (SEQ ID No. 33, amino acids 40-52),

genetically fused to a nucleic acid molecule encoding a carrier protein.

30 37. The expression system according to claim 36 wherein the carrier protein is a highly immunogenic carrier protein.

38. The expression system according to claim 37 wherein the carrier

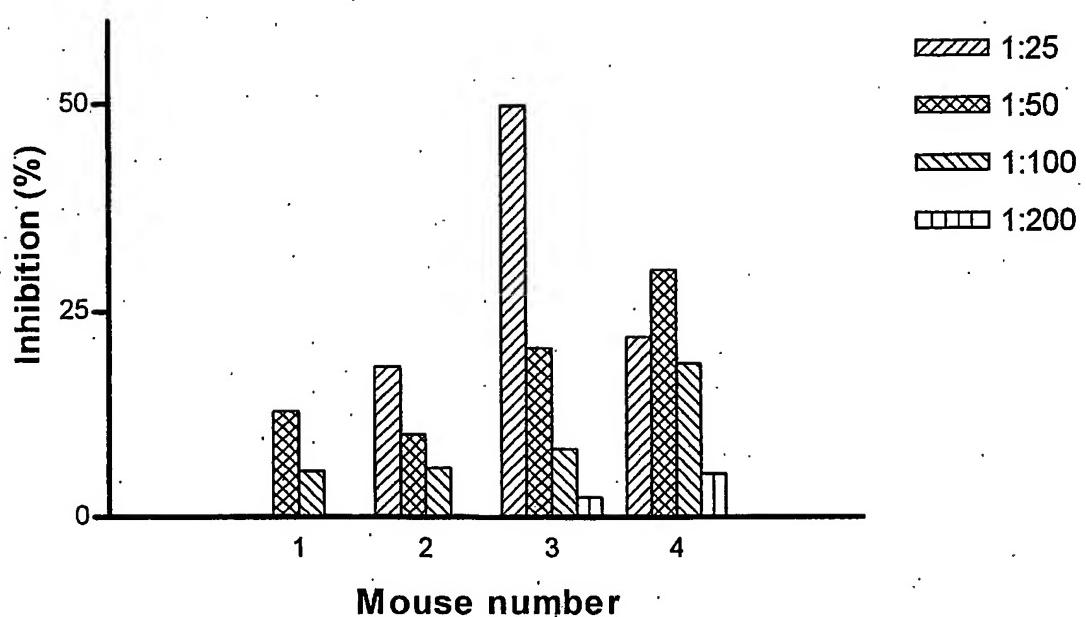
protein is selected from the group consisting of hepatitis B core antigen and hepatitis B surface antigen.

39. The expression system according to claim 38 wherein the peptide is fused into an immunodominant region of the carrier protein.

5        40. The reagent according to claim 1 wherein the chemokine is eotaxin and the peptide is selected from the group consisting of 6 or more consecutive residues of GPASVPTTCCFNLA (SEQ ID No. 30, amino acids 1-14 of eotaxin); 6 or more consecutive residues of FNLANRKIPLQRLES (SEQ ID No. 31, amino acids 11-25 of eotaxin); 6 or more consecutive residues of RITSGKCPQKAVIFKT  
10 (SEQ ID No. 32, amino acids 30-43 of eotaxin); and 6 or more consecutive residues of IFKTKLAKDICAD (SEQ ID No. 33, amino acids 40-52).

15        41. The method according to claim 12 wherein the chemokine is eotaxin and the peptide is selected from the group consisting of 6 or more consecutive residues of GPASVPTTCCFNLA (SEQ ID No. 30, amino acids 1-14 of eotaxin); 6 or more consecutive residues of FNLANRKIPLQRLES (SEQ ID No. 31, amino acids 11-25 of eotaxin); 6 or more consecutive residues of RITSGKCPQKAVIFKT (SEQ ID No. 32, amino acids 30-43 of eotaxin); and 6 or more consecutive residues of IFKTKLAKDICAD (SEQ ID No. 33, amino acids 40-52).

20        42. The method according to claim 24 wherein the chemokine is eotaxin and the peptide is selected from the group consisting of 6 or more consecutive residues of GPASVPTTCCFNLA (SEQ ID No. 30, amino acids 1-14 of eotaxin); 6 or more consecutive residues of FNLANRKIPLQRLES (SEQ ID No. 31, amino acids 11-25 of eotaxin); 6 or more consecutive residues of RITSGKCPQKAVIFKT (SEQ ID No. 32, amino acids 30-43 of eotaxin); and 6 or more consecutive residues of IFKTKLAKDICAD (SEQ ID No. 33, amino acids 40-52).

**IL-4 inhibition tests****Fig 1.**

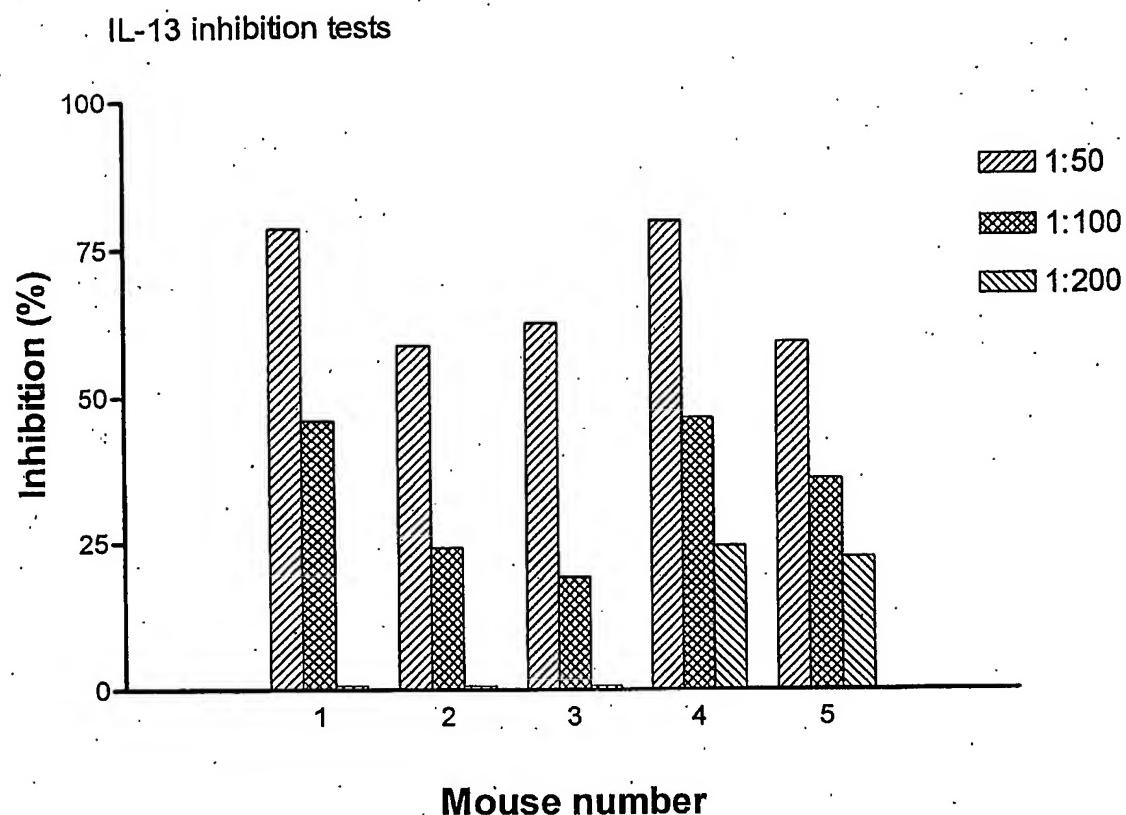


Fig 2.

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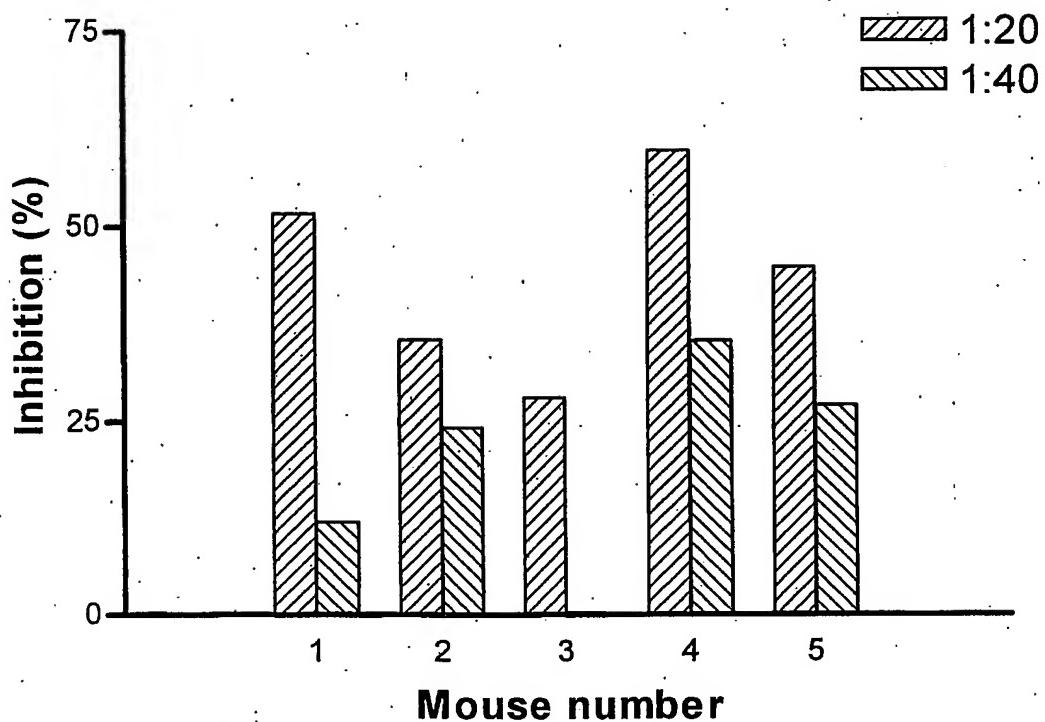
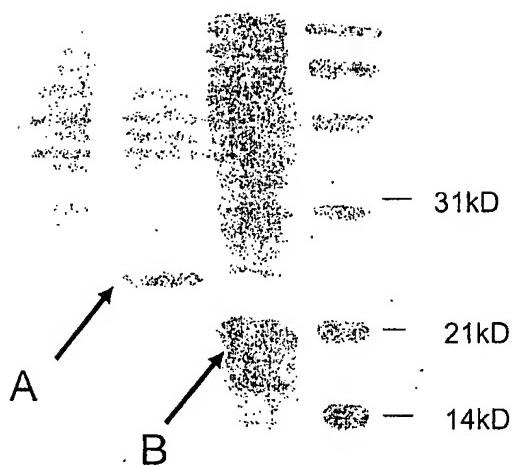


Fig 3.

## SDS-PAGE

1 2 3 4



### Immunoblot with anti-IL-4

1 2 3

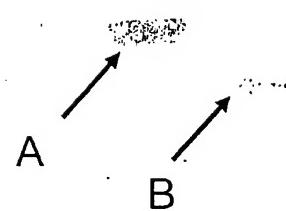
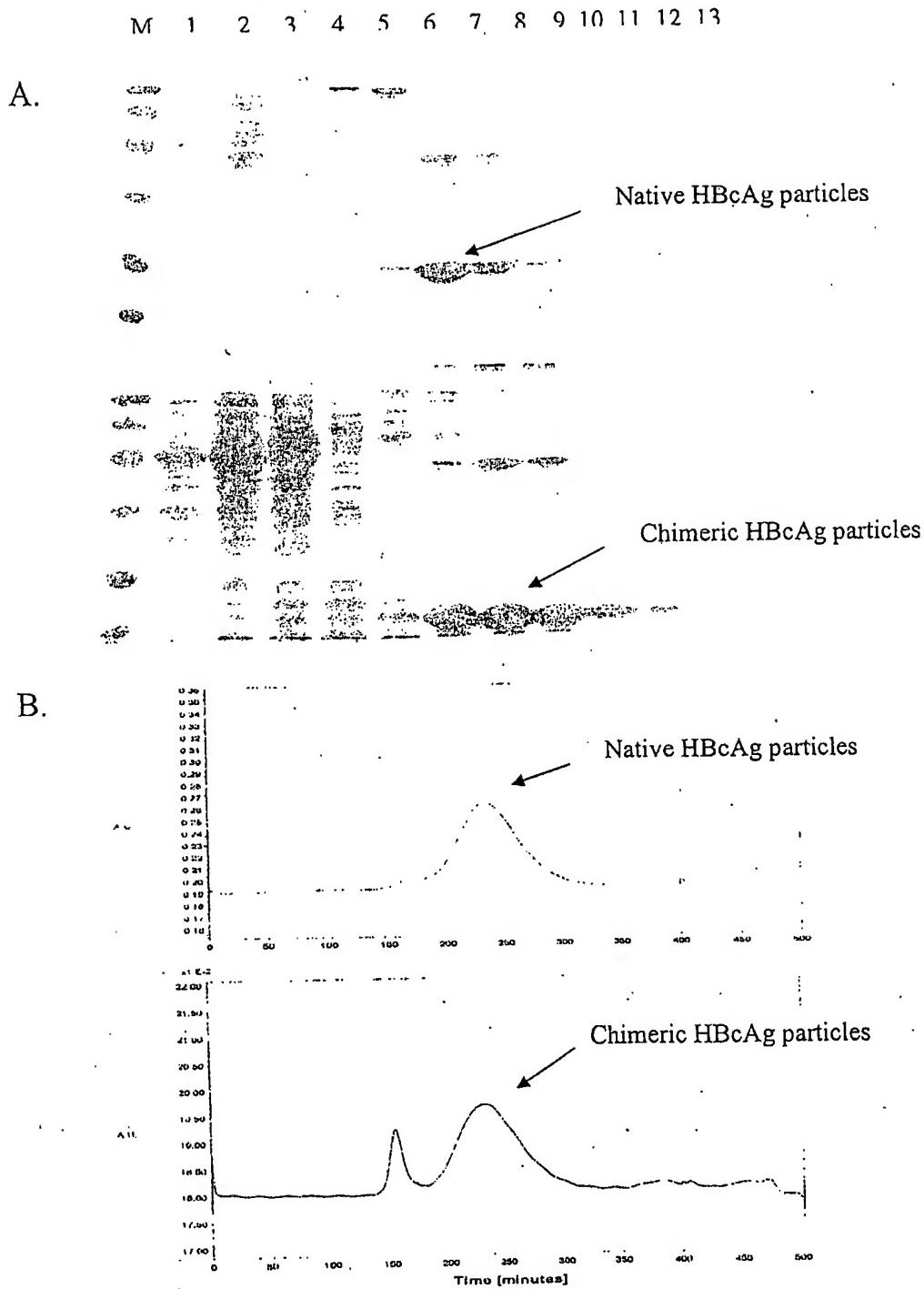


Fig. 4.



**Fig. 5.**

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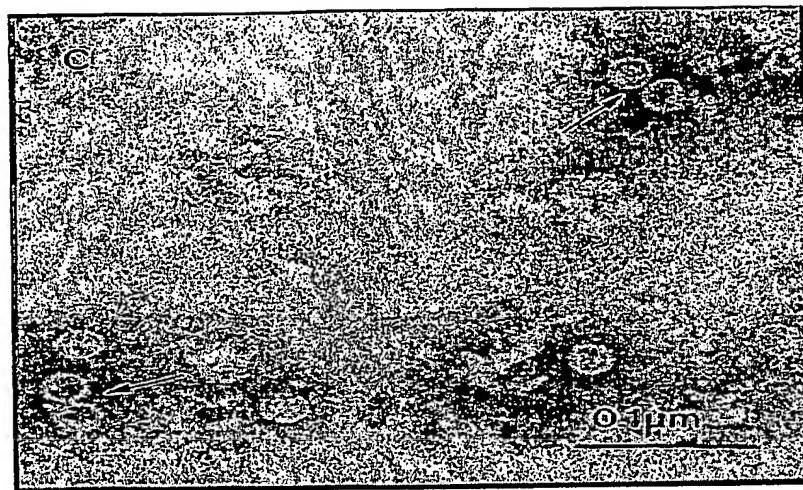


Fig. 6

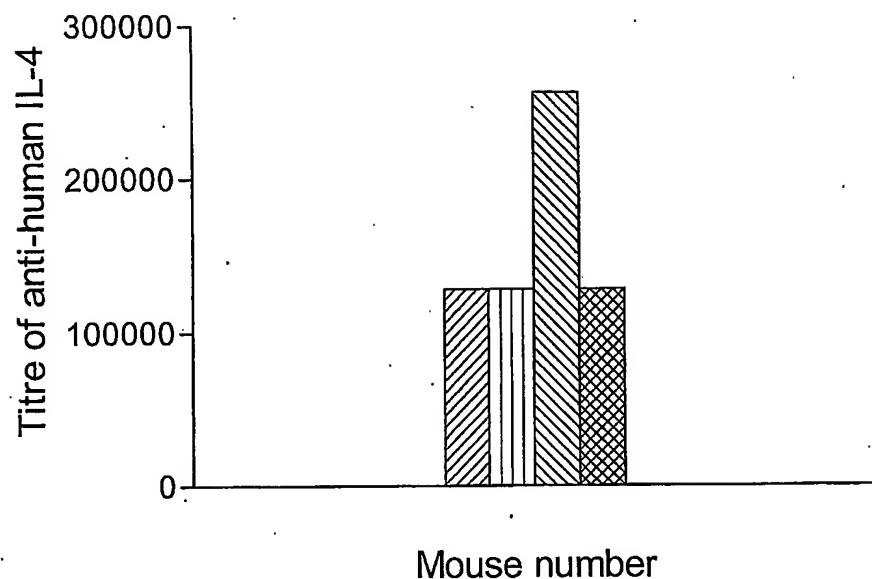


Fig.7.

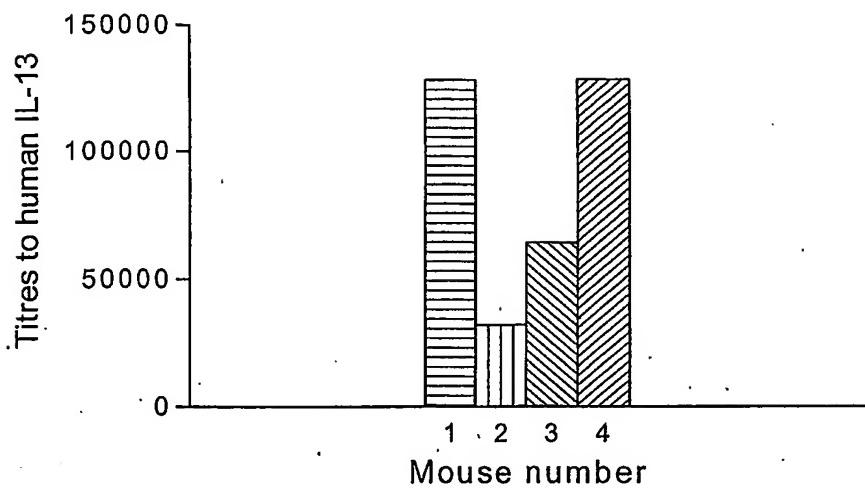


Fig.8.

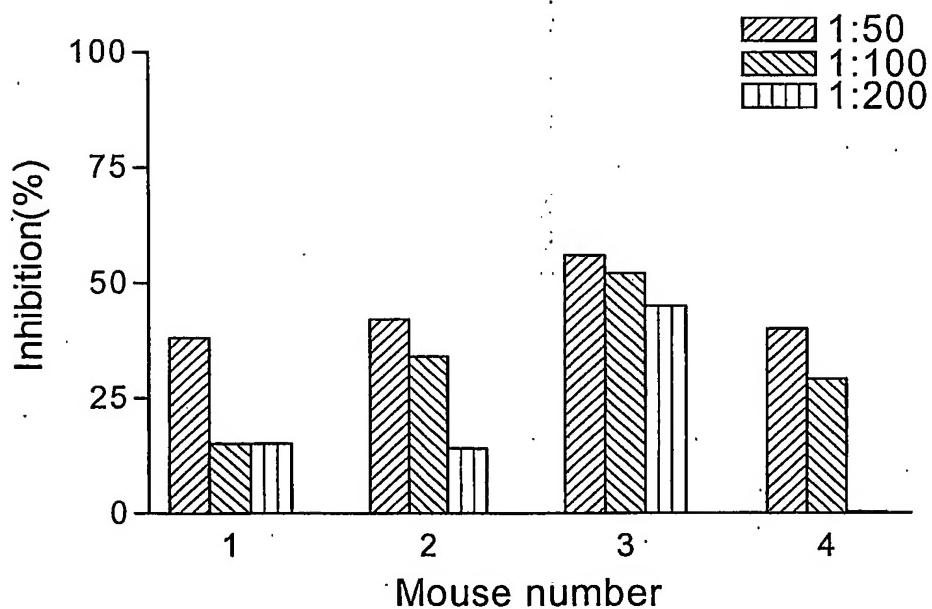


Fig. 9.

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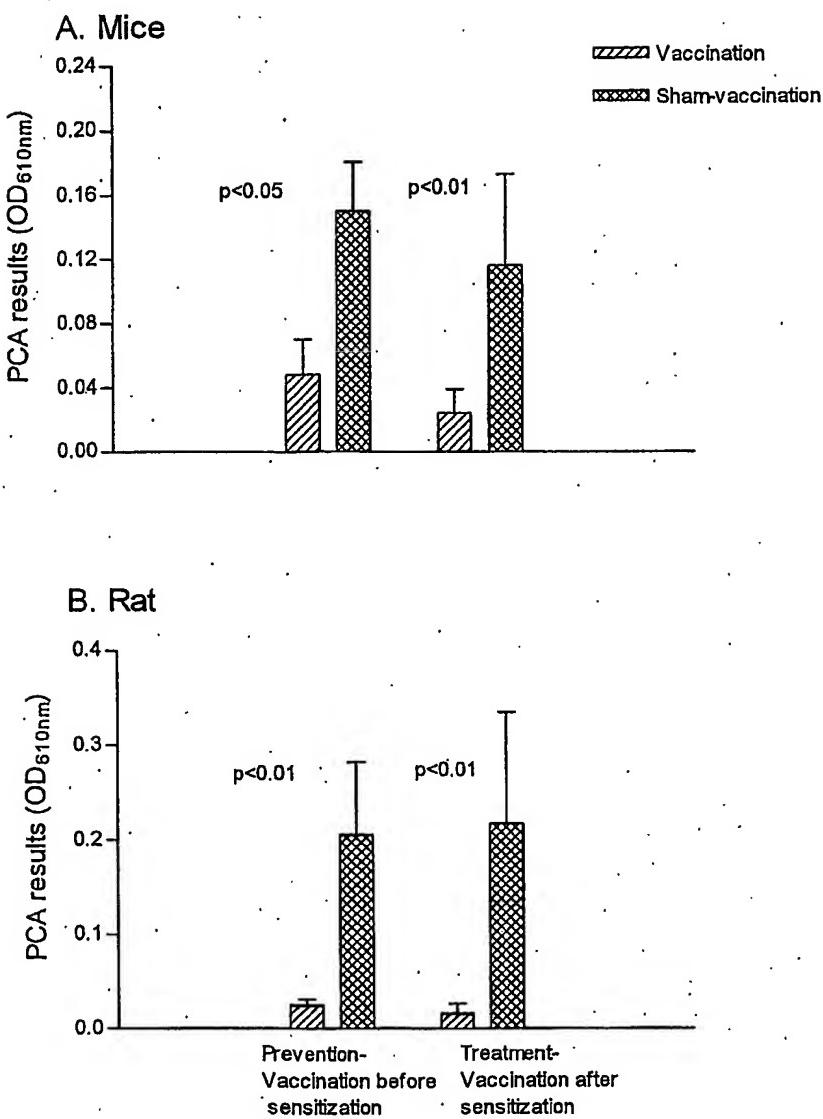


Fig. 10

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Manitoba Institute of Child Health  
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Peng, Zhikang

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